TOXICITY TEST FOR SOIL AND SEDIMENT USING *EISENIA* sp.

1. TEST OBJECTIVE

To assess the toxicity of a soil and/or sediment sample to *Eisenia foetida* (red worm) or other earthworm species and determine the effects on survival of the test organisms compared to controls.

2. TEST ARTICLE

2.1 Description/Identification

Unless otherwise specified, the test material is supplied by the client. The test article is a soil and/or sediment sample. Adequate chemical specifications with special reference to hazardous properties and storage conditions are also supplied by the client. When available, information on the stability, composition, or other characteristics which define the test article are on the file with the client.

2.2 Sample Preparation

Soil and/or sediment samples are collected based on project specific objectives. The samples are shipped on wet ice via overnight courier and stored in the dark at 4°C when not in use and evaluated a maximum of 2 weeks after sample collection. A minimum of 1 kg of sample is collected for each screening test. Minimal manipulation of the samples is performed in order to evaluate the sample in as close to in situ conditions as possible. Samples are carefully homogenized. Percent moisture of each sample is evaluated using ASTM (D421) standard guide. The percent moisture of the samples may be adjusted as appropriate to meet study objectives (e.g., 35-45% moisture or 75% of water holding capacity). The pH of the samples is determined and may be adjusted (depending on project requirements) using calcium carbonate and dilute mineral acid (HCl) and base (NaOH) if outside the pH range of ≥ 4 but ≤ 10.

An artificial soil/sediment or appropriate project specific reference site soil/sediment will be used to evaluate the effect of the individual samples to the worms. The artificial soil will be used as the control soil (laboratory control) following the formulation presented in EPA Region IV Standard Operating Procedures for the Toxicity Testing Hazardous Waste Assessment (Harris, et al. 1990). The following constituents will be mixed together on a dry weight basis to produce the artificial soil: 10 percent sphagnum peat moss (e.g., Maximillion Lenner Corp., New York, New York); 20 percent Kaolinite Clay (e.g., Burgess Pigment Co., Sandersville, Georgia); and 70 percent grade 70 silica sand (e.g., Valley View Farms, Sparks, Maryland). Addition of calcium

carbonate (approximately 0.4 percent) will be added to adjust to the pH range of 6.5±0.5.

3. EXPERIMENTAL DESIGN

3.1 Test Organisms

3.1.1 Species

The test species is the red worm *Eisenia foetida* or other earthworm species.

3.1.2 Source

E. foetida or other earthworm species used for toxicity tests are obtained from an organism vendor as specified in the report.

3.1.3 Culturing and Holding Conditions

E. foetida used in testing are purchased commercially. Organisms are gradually acclimated to testing temperature in an environmentally controlled laboratory at 20±2°C, with a 16-hour light, 8-hour dark photoperiod. If the organisms need to be held for several days, the cultures can be fed manure and/or baby food *ad libitum*. Worms are not fed during testing.

3.1.4 Age of Test Organisms at Test Initiation

Adult worms used to initiate testing are >300 mg (wet weight).

3.2 Test Concentration Series

E. foetida are exposed in replicate chambers to soil or sediment samples and to a laboratory or reference sample control. Screening assays may be conducted on whole soil or sediment samples. Alternatively, a definitive (multi-concentration test) may be conducted on a sample using a laboratory or reference soil/sediment to prepare the test concentrations.

3.3 Testing Exposure Design

Each sample is evaluated by placing a minimum of 200 g of test soil or sediment into each chamber. The test chambers are then placed in an environmentally controlled chamber at 20±1°C for the 14-day exposure period.

3.4 Test Vessels and Test Volume

Test vessels are 500-ml exposure chambers containing a minimum of 200 g of sample. The size of the test vessels and the volume of sediment may be changed depending on the study requirements.

3.5 Test Organism Number

Tests are conducted using a minimum of three replicates per sediment sample with ten organisms randomly assigned per chamber.

3.6 Test Environment

The test vessels are maintained in an environmentally controlled laboratory with continuous lighting. Temperature within the environmental room is monitored continuously using temperature recorders and is maintained at 20±1°C (unless a different project-specific temperature is required).

3.7 Test Observations

The organisms are observed daily and observations of non-burial are recorded. Any organisms which have crawled up the side of the exposure chamber are gently placed back onto the surface of the sample.

The study terminates after 14 days of exposure. At test termination, the number of surviving test organisms in each replicate are recorded. Death is defined by a lack of response to a gentle prod with a glass rod to the anterior of the test animal. Worms decompose rapidly in soil, and those organisms not found are presumed dead.

3.8 Data Analysis

Statistical analyses are performed on percent survival. For screening tests, a t-test is conducted to determine if a single test sample is significantly different from the control. For definitive assays, an analysis of variance (ANOVA) and either Bonferroni's T-Test or Dunnett's Mean Comparison Test are used to analyze significance of effects. Depending on the distributional characteristics of the data generated, it may be necessary to use Steel's Many-One Rank Test or the Wilcoxon Rank Sum Test instead (US EPA 1994). An LC50 and/or EC50 may be calculated from a definitive test using the probit, moving average, and binomial methods as described by Stephan (1977). Depending on the nature of the data, other methods may be used including the Trimmed Spearman-Karber method, the probit approximation method of Litchfield and Wilcoxon (1949), SAS probit analysis (SAS Institute 1985), or graphical interpolation using

the log concentration vs. percent mortality and/or percent affected as described by APHA et al. (1995). The statistical methods are specified in the final report.

4. FINAL REPORT

The final report is prepared to contain, at a minimum, the following information:

- Objectives and procedures stated in the approved protocol, including any changes made to the original protocol
- Identity of the test article(s) by name or code number and a description of any pretreatment
- Duration of the assay
- Survival observations recorded during the test
- Any unforeseen circumstances that may have affected the quality or integrity of the study
- Signature of the project manager, senior technical reviewer, and quality control officer authorizing release of the report
- Location of all archived data and the original copy of the final report at EA

Items of data to be included in the report consist of experimental design and test performance, effects on general appearance of test organisms (if applicable), morbidity and mortality, tabular presentation and appropriate statistical evaluation of environmental parameters, and survival data.

5. QUALITY ASSURANCE

5.1 Amendments to Protocol

Amendments to the authorized protocol established by EA or by the client are made only after proper authorization. Such authorization is achieved by completion of the Protocol Amendment Form by EA after consultation with the client.

5.2 Standard Operating Procedures

Unless otherwise specified, all procedures given in the protocol are subject to detailed Standard Operating Procedures (SOPs) which are contained in the SOP manuals of the participating departments. These SOPs and protocols generally follow the type of requirements in the U.S. EPA's Good Laboratory Practice Standards (GLPs) (US EPA 1989).

5.3 Reference Toxicant

A reference toxicant test, utilizing 2-Chloroacetamide, or another appropriate chemical is used as an internal quality check of the sensitivity of the test organisms. Testing is conducted on each population of organisms purchased for testing from an outside source if reference toxicant data are not available from the supplier on the acquired lot. The tests are performed as a 48-hour filter paper contact test. The results of each test are compared with historical, species-specific toxicological information from reference toxicant tests performed at EA, to determine if the results are within acceptable limits. Limits are established using the control charts outlined in US EPA (1993).

5.4 Quality Assurance Evaluation

Studies conducted under this protocol may be subject to internal audit by EA's Quality Assurance Unit. A quality control officer is responsible for monitoring each study to assure the client that the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with EA's QC program and, if applicable, EPA's GLPs.

5.5 Inspection by Regulatory Authorities

In the event of an inspection of EA by an outside authority during the course of the study, the client whose study is being inspected will be consulted before examiners are permitted access to any of the project records or the experimental areas.

5.6 Archives

Copies of project-specific records shall be transferred to the client promptly after the project is completed or as negotiated and budgeted. Original primary data are retained at EA for 5 years. Primary data include chain-of-custody records, laboratory data sheets, records, memoranda, notes, photographs, microfilm, and computer printouts that are a result of the original observations and activities of the study and which are necessary for the reconstruction and evaluation of the study report.

5.7 Location

Studies are conducted at the Ecotoxicology Laboratory of EA Engineering, Science, and Technology, Inc. at the Loveton Office in Sparks, Maryland.

6. SPECIFICATIONS OF THE *Eisenia* sp. SOIL/SEDIMENT TOXICITY TEST

6.1 Basic References

American Public Health Association (APHA), American Water Works Association, Water Environment Federation. 1995. Standard Methods for Examination of Water and Wastewater, 19th or most recent version. APHA, Washington, D.C.

American Society for Testing and Materials (ASTM). 1995. Standard Guide for Conducting a Laboratory Soil Toxicity Test with Lumbricid Earthworm *Eisenia foetida*. ASTM Designation: E1670-95, Philadelphia, Pennsylvania.

Athey, L.A., J.M. Thomas, J.R. Skalski, and W.E. Miller. 1987. Role of Acute Toxicity Bioassays in The Remedial Action Process at Hazardous Waste Sites. U.S. Environmental Protection Agency, Corvallis, Oregon. EPA/600/8-87-044.

Callahan, C.A., L.K. Russell, and S.A. Peterson. 1985. Comparison of Three Earthworm Bioassay Procedures for The Assessment of Environmental Samples Containing Hazardous Wastes. U.S. Environmental Protection Agency, Corvallis, Oregon. EPA/600/J-85/447.

EA. 1996. Quality Control and Standard Operating Procedures Manual for EA's Ecotoxicology Laboratory. Fifth Revision. EA Manual ATS-102. Internal document prepared by EA's Ecotoxicology Laboratory, EA Engineering, Science, and Technology, Inc., Sparks, Maryland.

Green, J.C., C.L. Bartels, W.J. Warren-Hicks, B.R. Parkhurst, G.L. Linder, S.A. Peterson, and W.E. Miller. 1989. Protocols for the Short-Term Screening of Hazardous Waste Sites. EPA 600/3-88/029.

Harris, T., J. Glover, and J. Maudsley. 1990. Region IV Standard Operating Procedure for Toxicity Testing Hazardous Waste Assessments. U.S.EPA. Region IV, Athens, Georgia.

Litchfield, J.T., Jr. and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharm. Exp. Ther. 96:99-113.

Peltier, W.H., and C.I. Weber. 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. EPA/600/4-85/013. U.S. Environmental Protection Agency. Cincinnati, Ohio.

SAS Institute Inc. 1985. SAS User's Guide: Basics, Version 5 Edition. Cary, NC: SAS Institute Inc. 1290 pp.

Stephan, C.E. 1977. Methods for calculating an LC50, in Aquatic Toxicology and Hazard Evaluation (F.L. Mayer and J.L. Hamelink, eds.), pp. 65-84. ASTM STP 634. American Society for Testing and Materials, Philadelphia, Pennsylvania.

US EPA. 1989. Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards. Title 40 CFR Part 792. Fed. Regist. 54(158): 34034-34074.

US EPA. 1989. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Second Edition. EPA/600/4-89/001. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.

US EPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fourth Edition. EPA/600/4-90/027. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.

US EPA. 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fourth Edition. EPA/600/4-90/027F. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.

US EPA. 1994. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Third Edition. EPA/600/4-91/002. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.

6.2 Test Specifications

Test organism:	*Eisenia foetida* or other earthworm
Organism age:	Adult (>300 mg wet weight)

Test Type:	Static
Temperature:	20±1°C
Light quality:	Wide-spectrum fluorescent light
Light intensity:	50-100 f.c.
Photoperiod:	Continuous
Test container:	500-ml (minimum) glass wide-mouth jars
Test soil/sediment mass:	200 g (minimum)
Test soil pH:	≥4 but ≤10 (s.u.)
Artificial soil laboratory control (% weight):	10% sphagnum peat, 20% colloidal clay, 70% silica sand
No. of replicates:	A minimum of 3 replicates
No. organisms per container:	10
Feeding regime:	Organisms not fed during testing
Test duration:	14 days
Endpoints:	Survival
Test acceptability:	May vary depending on regulatory requirements; one criterion is 80 percent or greater survival in all control replicates